MANAV RACHNA INTERNATIONAL UNIVERSITY

Short Synopsis
For
Ph. D. Programme 2010-11

DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF ENGINEERING & TECHNOLOGY

MANAV RACHNA INTERNATIONAL UNIVERSITY

SECTOR-43, SURAJKUND –DELHI ROAD, FARIDABAD – 121001

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TITLE: Screening Of Azolla microphylla Biomass for its Biosorption Potential and Removal Of Heavy Metals: Cadmium And Lead From Waste Water

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ABSTRACT

Pollution is increasing day by day due to increase in population, urban growth and industries. Both organic and inorganic pollutants are increasing in the air, soil and water. These pollutants directly or indirectly through food chain are entering into the human bodies. Among the various pollutants heavy metals are most hazardous as they do not degrade. They bioaccumulate and hence bioconcentrate in the food chain. Different methods are used to get rid of pollutants. Phytoremediation is a novel, eco-friendly, economical method to remove heavy metals from environment by the help of plants. *Azolla microphylla* is an aquatic heterosporous pteridophyte with many advantages. It is globally distributed, less doubling time, greater biomass, easy to harvest, can fix atmospheric nitrogen due to its association with cyanobacteria *Anabaena azollae.* In the present study attempt will be made to explore the ability of *Azolla* for the biosorption of Cadmium and lead in the effluent generated by the industries in Faridabad and dispersed in Buria Nalaha which ultimately falls in river Yamuna. For this, chemical characteristics and heavy metal profile of water of Buria Nalaha, Faridabad would be studied for twelve months using the standard methods approved by APHA (American Public Health Association) and CPCB (Central Pollution Control Board). The growth behaviour of *Azolla* species exposed to heavy metals Cadmium and Lead and Buria Nalaha effluents will be studied with help of standard protocols mentioned later in the research plan. The antioxidant enzymes in *Azolla* plants under heavy metal stress will be evaluated and the physiological mechanism of adaptation to heavy metal toxicity will be elucidated. If time permits the biosorption capacity of Cadmium and Lead by *Azolla*. The observations and the result will approve the remediation capability of Azolla with reference to Cadmium and Lead from waste water.

Keywords: Pollution, Heavy metals, Phytoremediation, Azolla microphylla, Antioxidants.

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TITLE: ***Screening Of Azolla microphylla Biomass For Its Biosorption Potential And Removal Of Heavy Metals: Cadmium And Lead From Waste Water*.”**

INTRODUCTION:

Environmental pollution is increasing due to increase in urbanization, exploding population and industrialization. Toxic wastes originate from industrial and domestic operations. These wastes are released into the important compartments of our environment i.e. air, soil and water. Accumulation of these toxic wastes in environment is of great concern, as they are hazardous to health by making entry through food chain. These hazardous substances include both organic and inorganic compounds. Radioactive elements, heavy metals, trace elements and metalloids, are the inorganic substances that find entry by different geophysical modifications like mining etc.

Use of insecticides, herbicides, fungicides in agricultural practices and many other chemicals in our day to day life add the harmful organic substances. Natural precipitation and inorganic salts accumulation in soils make them saline or sodic, thus rendering agricultural soils as unproductive. Modern agriculture requires intensive irrigation and many pollutants are added to soils with irrigation waters. Factors like precipitation, specific as well as non-specific absorption determine the fate of toxic substances in soil and aquatic environment like non-specific, dissolution, photochemical reaction, biotic processes etc. further decide the fate of these harmful substances (Huang 1993)

When these pollutants cross a threshold limit their removal becomes essential. Chemical processes being used the waste treatment have their own constraints. Hence, new biotechnological methods are being applied for waste treatment. In these methodologies living organisms are used to remove harmful compounds. In bioremediation lower organisms (algae, bacteria and fungi) are used while use of higher plants for treating polluted soil and water is termed as phytoremediation. Both these technologies have the advantage of ex-situ, in-situ and in-vivo application and are environmentally acceptable in comparison to the other physico-

chemical approaches (Pletsch et al. 1999). Polluted soils are treated and reclaimed by use of higher plants i.e. phytoremediation, while aquatic systems are treated using small water plants and microorganisms. Few aquatic plants which have been used for wastewater treatment are *Azolla, Lemna, Eichornia* etc.

Heavy metals are considered to be most hazardous substances present in our environment, as they are highly durable and most harmful to living organisms. Biosorption is the sequestering the heavy metals present by the use of microorganism or green plants. It is a novel technology to overcome the harmful conditions due to heavy metal pollution. In phytoremediation, the plant roots collect the pollutants and then decompose them to less harmful substances or accumulate them in their tissues. This technique is not only cheap but also eco-friendly. In addition decomposition product does not require to be used further (Sternberg et al. 2002, Roy et al. 1992).

Generally two mechanisms are involved to remove metals present in water with the help of aquatic plants. One is an independent quick surface reaction metabolism which occurs within minutes like diffusion and ceases on binding of dissolved metal ions to the outer surface of cell wall. The other metabolic process takes hours or even days. It is a dependent process and inlvolves uptake by the cell mass and its transfer into inside of cell (Axtell *et al.* 2003, Cho *et al.* 1994).

Use of live cells has advantage over non-living ones for removing hazardous metals because live cells perform similar to non-living mass at low metal concentrations and they also reproduce generating more of itself thus removing metal by second mechanism. But according to Wang and Wood(1984), use of non-living biomass is desirable in case when heavy metals is present in high concentration because these metals are highly toxic to organism.

Azolla, a water fern which is free floating, and has symbiotic association with *Anabaena azollae,* a blue green alga that can fix N_2 from atmosphere. It can reproduce at a very fast rate (time to double itself is around 2-3 days), has a high capacity for N_2 fixation which builds up nitrogen content and high protein content. So, it is used in fields growing rice as biofertilizer rich in nitrogen..

Azolla is distributed worldwide as it can grow in different conditions from fresh water to polluted waterbodies (Singh and Mahapatra, 2000). It has the ability to take up nutrients directly from flood waters as it has high affinity for K, Fe and P. Nutrient accumulation during life time is many times higher than required which are released back slowly into environment on decomposition of its dead biomass. It is used in treatment of waste waters owing to its capability to concentrate essential nutrients like N, P and non-essential heavy metals. (Wagner 1997).

It has been found that some species of *Azolla* can grow in fairly high salt concentrations. Hence it can be utilized for reclamation of saline soils particularly in coastal areas. *Azolla* has high possibility for being used in phytoremediation of polluted soils as well as wastewaters. *Azolla* can be used

a) For treating wastewaters having high content of heavy metal.
b) Treating P and N rich effluents like domestic sewage.
c) Phytoremediation of soils having high salinity.

Azolla has distinctive capability for absorbing heavy metals like chromium, nickel, cadmium zinc, copper and uranium. In a study conducted by Yong-Huang and Weizhen in 1985 on four *Azolla* species for their tolerance to Mn, Cu, Zn, Fe, Mo, Cd, Co etc under laboratory conditions, it was found that growth of *Azolla* was hardly affected due to its concentration capacity suggesting possible use of *Azolla* in sequestring heavy metals from polluted waters. Thereafter, *Azolla* has been studied either in mixed culture or alone for treating wastewaters.
Removing metal from effluents using *Azolla* is advantageous as compared to conventional methods because

a) Presence of environmental and ecological hinderances
b) Very low concentration of heavy metals but higher than limits permitted (i.e. 1-20 ppm). In these cases chemical methods of metal ion removal are ineffective.
c) Harvesting biomass of *Azolla* is very convenient as it is freefloating .

Removing heavy metal by *Azolla* can occur by following manner -

1. Active mechanism,

2. Passive mechanism

In first process, *Azolla* plants is cultured in tanks/ponds having wastewater from which heavy metal is to be removed, while in second process the filters packed with dry biomass of *Azolla* are used ,through which are effluents are passed. Both these processes have proven to be effectual in treatment of polluted/ brackish water. *Azolla* is more suitable for use in wastewater treatment than rooted aquatic plants as it is free floating.

Recently, new ahievements have been seen in the area of heavy metals exclusion using passive processes by means of biomatrix or biofilters made from *Azolla*. Its biomatrix has the possibility to be used for concentrating precious metals and removing hazardous heavy metals. The major advantages of using biomatrix processes are:

1. *Azolla* biofilters can readily bind metals to a very high extent i.e. Pb:10% > Cr^{3+}:9% > Cd :3.7% > U :3.5% > Ni:3% > Cr^{6+}:2% .
2. Effluents loaded with heavy metals can be purified to very low conc. in a single step.
3. *Azolla* biofilters retain their capacity to bind metals at different pH ranging between 3.0-11.0.
4. Metal binding capacity does not decrease after washing with acid.
5. Its biomass can be conveniently produced in outdoor farms and polyhouses.
6. *Azolla* biomass obtained after heavy metal removal can be decomposed.
7. Efficiency of *Azolla* is more as compared to that of active charcoal, zeolite, active earth, etc.
8. Presence of pectin in high amount forms proper matrix and its less cellulose allows easy flow of solution.
9. Radioactive wastes can also be detoxified easily by *Azolla*.

Cadmium is an important environmental contaminant not only because of its presence and bioaccumulation introduces it to other organisms in the the food chain. Cd is introduced in the atmosphere through power plants (coal-fired), steel mills, metal smelters and roasting operations, waste inceneraters, and electroplating industries (Rauf, Ubaidullah and Abdullah,2009).

Experiments carried under controlled laboratory conditions indicate that cadmium may function in and may be an important factor for different pathological processes which include tumors of testis, kidney disorders, hypertension, arteriosclerosis, inhibition of growth, chronic diseases associated with old age, and cancer. Also, it was reported that cadmium may be responsible for lesions within the central nervous system in sensory ganglia that are haemorhagic (D. F. Flick et al., 1971). In plants, cadmium at higher concentrations reduces chlorophyll production as shown in *Azolla* (Daniel Gonzalez-Mendoza et al. 2011).

High levels of lead have been reported to cause several health problems which include anemia, kidney disorders, liver malfunction, paralysis, damage to brain, convulsions and at timescan be fatal. Low concentration of lead can result in hyperactivness, learning disorders in children, night blindness and the suppressing the immunity of body (Jain et al. 1990). Lead is therefore a potent environmental pollutant and health hazard which is discharged into the water system by, for example many battery manufacturing plants.

This study attempts to explore the ability of Azolla for the biosorption of Cadmium and lead in the effluent generated by the industries in Faridabad and dispersed in Buria Nala which ultimately falls in river Yamuna

LITERATURE REVIEW:

The ability of the plant materials to remove toxic metals form solution has been reported by several researchers. *Azolla* is a widely distributed small water fern. It is found to have symbiotic association with heterocystous *Anabaena azollae*, a blue green alga, which is able to fix nitrogen from the air. This allows the ferns to grow well in water bodies that lack nitrogen and not suitable for survival of aquatic plants. *Azolla* species has been classified as weed due to its capacity to form dense mat which is a cause of concern to naturally occurring ecosystems and restrict free flow of water and limits open water areas required for recreational purposes. The use of *Azolla* species biomass as a cheap and readily available material for possible application in waster water remediation has been studied by many workers.

Azolla filiculoides biomass (non-viable) has been reported to remove lead from solution by 93mg of lead per gram of plant by **Sanyahumbi Douglas et. al (1998)**. Concentration of lead removed varied at different pH, at pH 1.5 from of the initial 30% lead concentration and approximately 95% at pH range values of 3.5-4.5. At initial 400 mg/l lead concentration, 30% decrease in lead removal was observed. 90% removal of lead occurred in range of 10 °C to 50 °C. Concentration of biomass hardly effected removal of lead.

Ganji M.T. *et.al* (2005) worked on *Azolla filiculoides* to study the biosorption by treated *Azolla* to remove lead, cadmium, copper and zinc from the wastewater. Heavy metals adsorbed on $H_2O_2/MgCl_2$ treated *Azolla filiculoides* .Under stable conditions for zinc, copper cadmium and lead ions the maximum absorption capacities of the *Azolla* gathered from rice field were approximately 48, 62,86 and 228 mg/g respectively in dry biomass,whereas it was about 34, 33, 58 and124 mg/g respectively in *Azolla* from the Anzali International at the same condition. Such reduction in absorption is due to the contamination of wetland. HCl and NaCl desorbents were used for recovery of heavy metals which was 64- 86% and 51-72% respectively.

Vasudevan P. et.al (1990), compared uptake capacity of *Lemna minor* and *Azolla pinnata*. Different concentration 8.0 mg/l, 4.0, 2.0, and 1.0 mg/l were taken and the metals were replenished on every second day for a period of 14 day. At the concentration of 1.0 mg/1 the highest metal absorption was observed. With time the amount of lead/ zinc left behind increased when water velvet /duckweed was given treatment at 1.0 as well as 2.0 mg/l levels. But at concentration of 4.0 ppm and 8.0 ppm, the amount of lead / zinc left behind showed varied results with the passage of time. At first it showed rapid increase for 8-10 days and after that it became constant. The rate of absorption of one metal was affected by that of the other's presence ; e.g. *Azolla* in only lead solution at 8.0 ppm, the bioconcentration factor was 54.5. But at equal concentrations of zinc and lead the concentration factor 35.44 of lead was observed, pointing towards the affect of the zinc ion presence on lead ions.

Lead and Cadmium removing capacity of *Azolla caroliniana* from wastewaters was investigated by **Stêpniewska Z. et.al (2005)** The study verified the capability of *Azolla caroliniana* to remove Pb and Cd from water. *A. caroliniana* was cultured in water having lead and cadmium concentrations of 0.1ppm, 0.5ppm and 1ppm each. The *A. caroliniana* growth was inhibited by

about 30-37 and 24-47%, respectively. The metal content in the water medium decreased by 90% in case of Pb(II) and 22% in case of Cd(II). 416 mg Pb per kg dry mass and 259 mg Cd per kg dry mass was found.

Azolla species:*A. microphylla, A. pinnata* and *A. filiculoides* were studied by **Arora A. et.al (2006)** to investigate the tolerance to chromium and extent of its accumulation and bioconcentration. In comparison to controls, above species when grown in presence 10 µg/ ml Chromium concentration showed 30–70% biomass production No effect on activity of nitrogenase was seen at 1–5 µg /ml conc. but it is highly reduced at higher concentrations. Growth was not observed at high chromium concentration. But the plant showed necrosis at high chromium concentration and accumulation of Cr to about 5000–15,000 µg g^{-1}.With increase in chromium level , metal present in the dry biomass increased. Bioconcentration Factor (BCF) was greatest for *A. microphylla* i.e.4617. Hence these *Azolla* spp. can be usedfor treating effluent from tannery and other wastewaters having Cr.

Physiological responses at different concentrations of mercury were studied by **Bennicelli R.P. et.al (2005***)* in *Azolla caroliniana* Willd. for 9 days in laboratory. *Azolla caroliniana* was grown on culture medium having1, 20 and 30 mg Hg (II) dm^{-3}. During this duration the superoxide dismutase activity and oxygen diffusion rate were observed. Oxygen release by roots of the fern was strong around 50 µg $m-^{2}s^{-1}$ and is correlated with SOD activity increase which was about 4000 units more than value of control . This shows the presence of mechanism of defense in *A. caroliniana* in the presence of stress due to heavy metal.

The water soluble fraction (WSF) of crude oil of Olomoro was investigated for its characteristic both physical and ionic by **Noyo et al.(2008)** The WSF was exposed to *Azolla africana* Desv. The values were investigated at water soluble fraction of 25, 50 and 100%.The results showed that ion availability increased after the exposure to the test plant. *A. Africana* and there was decrease in the population of the macrophyte. These changes indicate that due to ionic stress there is leakage of ions from cells resulting in plant degradation. After macrophyte exposure conc. of Mg^{++}, NO_3^-, V and Cl^- ions in the growth medium decreased. This indicates uptake of these ions. It was observed that after plant exposure Mg accumulates in the tissues at 25 and 100% WSF.

In Transvaal, South Africa a wetland ecosystem polluted by effluents from mines, industries and sewage works was studied for Iron, Nickel, lead, Copper, Zinc, Chromium and Manganese by *Azolla filiculoides* by **Wet, et.al(1990)**. Different metals accumulated by the water fern reflected concentrations in the bottom sediments. Mn and Fe showed the greatest concentrations in *A. filiculoides* (μg dry mass). Calculated bioaccumulation factors indicated that Mn, followed by Pb, Ni and Cu were best absorbed by *A. filiculoides***.**

Rai and Tripathi (2009) compared potential of *Vallisneria spiralis* and *Azolla pinnata* for removing mercury from waters. The plants were grown for six days in media having Hg at different concentrations of 0.1, 0.5, 1.0 and 3.0 ppm. Purification potential of *V. spiralis* (70–84%) was less than*A. pinnata* (80–94%) . Hence more mercury was present in dry mass of *A. pinnata*. RNA, chlorophyll a, protein, DNA and nutrients uptake showed concentration dependent decrease in both due to toxicity of mercury. The decrease was less in *Vallisneria* than in *Azolla*. The results suggested that both the plants can be used to purify the industrial effluents containing Hg.

Bennicilli R. et al.(2004) studied the capability of *Azolla caroliniana* for heavy metal removal in water from municipal waste. He investigated the potential of *Azolla caroliniana* Willd. to remove mercury, and chromium (both Cr(III) and Cr(VI)) from polluted waters. Nutrient solutions having concentration 0.1, 0.5 and 1.0 ppm each of Hg^{2+}, Cr^{3+} and CrO_4^{2-} ions, were taken and for *Azolla* was cultured in it12 days. 20–31% growth inhibition was observed which was maximum for Hg (II) ions than control. In solution the metal content was reduced to 0–0.25 ppm after day 12, and the reduction in the metal content 1.0 mg dm^{-3} for Cr^{3+} was between 74 and for CrO_4^{2-} 100% 0.1ppm.Uptake of metal was less from 0.1 ppm than from 0.5 and 1.0 ppm solutions. Heavy metal concentration in the *A. caroliniana* tissues was found to be maximum in Cr (III) containing nutrient solution whereas it varied from 71 to 964 mg kg^{-1} dm;**.**

Ena Alba et al. (2007) investigated *Azolla* ability to remove polyphenols as well as chemical oxygen demand from wastewater of olive mill. They investigated biofiltration by *Azolla* by studying chemical oxygen demand (COD) removed along with quantity of polyphenols using

olive mill wastewater (OMWw) which was collected by the continuous extraction system (CS) and traditional systems (TS). Five sequential columns and five sequential Imhoff cones packed with *Azolla* biomass were used for experiment. In both cases, the filtrates from the last biofilter (5^{th}) showed reduction in content of polyphenol i.e. 3610 mg l^{-1} from 7650 mg l^{-1} in traditional system OMWw and 1351 mg l^{-1} from 3852 mg l^{-1} in continuous extraction system OMWw. The COD contents decreased to 52400 mg l^{-1} from 110200 mg l^{-1} in traditional system OMWw and to 2300 mg l^{-1} from 41600 mg l^{-1} in continuous extraction system OMWw. A ratio of 5:1 OMWw to *Azolla* fresh-weight was best for removing both polyphenol and COD.

It was found by **Khosravi et.al (2005)** that treated but intact biomass has the potential to get rid of heavy metals from polluted water . They took semi-intact, activated, and inactivated *Azolla filiculoides* and studied its capacity to remove lead, cadmium,nickel and zinc ions . The metal ion uptake by *Azolla filiculoides* that was activated by use of NaOH at pH 10.5 ± 0.2 , $CaCl_2$/$MgCl_2$/ NaCl with 2M concentration at 2:1:1 mole ratio, was found out to be about 271, 111, 71 and 60 mg/g respectively. It was observed that *Azolla* when was pre-treated with $AlCl_3$,HCl, $FeCl_2$, CH_3OH, $SrCl_2$, C_2H_5OH, and $BaCl_2$ the capacity for removal of the heavy metals decreased as compared to the semi-intact *Azolla*. The heavy metals uptake was slower in the semi-intact *Azolla* than by activated *Azolla*.

Zhao M. et.al (1999) in batch and column experiment on *Azolla filiculoides* investigated the capacity to remove zinc ions from electroplating rinse effluent. In batch systems highest zinc uptake by *Azolla* at pH of 6.0 was observed to be 45.2 mg/g. Good mechanical stability and flow-permeability was shown by *Azolla* even when column operations were repeated. Bounded zinc was completely desorbed with use of 120 ml of 0.2 N H_2S0_4 and HCl. The process was repeated for six cycles and the regeneration efficiencies suggest that *Azolla* can be reused in the treatment of waste water having Zn^{2+}.

Biological response of *Azolla filiculoides* Lamk in presence of Pb, Hg in water was investigated by **Anzhi R, et.al (1996)** . His observations indicate that the purifying potential of *Azolla filiculoides* Lamk for Pb is more than to Hg. Pb was lethal at15ppm and Hg at 10ppm respectively. The ability to purify water depends on kind of metal, its concentration, type of

tissue, growth period and quantity of *Azolla*. Pb and Hg decreases the growth of *Azolla*, the decreases chlorophyll and soluble protein content.

The response of *Azolla pinnata* to three different pesticides in terms of growth and NPK was investigated by **El-Shahate et.al (2011)**. Herbicide Saturn, insecticide furada and fungicide hinosan which is usually applied as a biofertilizer in rice, at varying concentrations, were selected. Different effects of the three pesticides were observed. In the presence of furadan and hinosan increase in growth rate of *A. pinnata* at lower concentrations of 0.001 and 0.002 ppm was observed along with increase in content of NPK. Nitrogenase activity at the different incubation periods was maximum at furadan concentration of 0.002 ppm. With increase in the incubation period of the applied hinosan and furadan, (incubation period of 20 and 25 days) the uptake of nitrogen, phosphorus and potassium increased at all concentrations being highest at the 0.002 ppm for both hinosan and furadan. But, saturn showed negative impact on growth, N2-fixation and the NPK content even at lowest concentration.

Biosorption potential of *Azolla filiculoides* to absorb Cesium and Strontium were studied by **Mashkani and Ghazvini.(2009)**. Results showed inhibition of 27.4% and 46.3% growth in case of presence of Cs and Sr respectively in culture medium. *A. filiculoides* when treated with 2 M $MgCl_2$ along with 30 ml H_2O_2 8 mM for 12 h at pH 7 followed by washing by NaOH solution for 6 h at pH 10.5 showed maximum removal of caesium and strontium ions. Modification of the surface characteristics by pretreatment improves biosorption process.

Effect of salinity levels on growth and induction of tolerance to high salt content in *Azolla pinnata* was studied by **Rai and. Rai 1999).** It was observed that *Azolla pinnata* could not tolerate an NaCl concentration applied externally more than 30 m*M*. However, on 18 days preincubation of plants in 20 m*M* of NaCl, followed by stepwise transfer in 10 m*M* NaCl per day, enabled them to survive at an otherwise lethal 60 m*M* NaCl concentration These rates could be compared to the growth in 20 m*M* NaCl. Adaptation involved developing capacity in the plants to regulate ion concentration.

Ghazvini and Mashkani(2007) worked on the removal of Cr(VI) and Pb(II) by *Azolla*. It was observed that pH of the solutions effect the biosorption potential, and it was maximum at pH 2

and 4 for Cr and Pb, respectively, Presence of different salt concentrations (NaCl) had no effect. *Azolla* biosorbed 43.7 and 70.5 mg/g dry weight of Cr (VI) and Pb9II) respectively, inone hour. For the first 5 min. absorption was fast and then it slowed till it reached equilibrium in 60 min. No significant change in uptake was found at temperature range of 25-45^0 C.Absorption increased with increase in biomass concentration while specific absorption decreased. Desorption of Cr(VI) and Pb(II) from *Azolla* was successful using 1mol/L NaOH and HNO_3 respectively, without causing any damage to biomass till 7 sorption –desorption cycles.

Awoden (2008) studied changes produced in physiochemical properties of soil by *Azolla* . In,an incubation experiment soil treated with *Azolla* at 0, 20, 40, 60, and 80gkg^{-1} was taken and incubated at field capacity in the absence of light for 48 days at 25^{0}C.It was found that after 8 weeks soil became more porous but its organic content, P, N, ,K, pH , Ca, Na and Mg elevated with increase in *Azolla* mass.

Zhang Xin et al.(2008) worked on *Azolla* to study As accumulation and tolerance. From the *Azolla* strains showing huge variation in amount of As accumulated. The strains at exhibiting extreme in absorption were selected for investigation. *Azolla filiculoides* showed more influx of arsenate and accumulated half As than *Azolla caroliniana*. Uptake of exterior As was less by *A.filiculoides* suggesting it was more resistant. Tolerance capacity to internal As was same for both the strains.

Arora and Saxena (2005) investigated the probability of using *Azolla microphylla* biomass in order to recycle municipal wastewaters. Wazirabad sewage treatment plant(New Delhi) was selected and municipal wastewaters was collected in all the four seasons to detect amount of reactive P and heavy metalconcentration. Presence of heavy metals like cadmium,lead, nickel, zinc, iron and manganese in the biomass were quite below permissible limits whereas 1-2 ppm P was detected .Possibility of growing *A.microphylla* on sewage effluent was high.

Growth rate with respect to temperature variation was studied by **Watanabe and Berja (1983).** From the four sp. selected , 22 strains of R.Br., 3 strain of *A. filiculoides* Lam. and one strain each of *A.caroliniana* Wild. And *A.mexicana* Presi were experimented separately for

growth studies.Controlled conditions with 12 hr photoperiod with artificial light 30klux was provided to growth cabinets. Different temperature levels 33^0C ($37/29^0$Cday/night),29^0C ($33/25^0$C) and 22^0C) were selected. With exception of *A.mexicana* strain and three all *Azolla pinnata* strains the growth rate increased at 33^0C as compared to that at 22^0C. In case of *A.filiculoides* and *A.caroliniana.* the later needs less temperature.for its growth.Among the four sp. *Azolla pinnata* was most tolerant.

Use of *Azolla* as biofilter for removing P from waste water was worked upon by **Costa et.al (1999).** *Azolla* growing in natural water ecosystems and in wastewaters was compared for its composition of its biomass and growth characters. Growth rate(0.107 ±0.037 per day) and productivity(5.8g dry wt.$m^{-2}d^{-1}$) confirms that *Azolla* can be grown in domestic wastewater partially treated to purify it , but not diluted pig waste..

OBJECTIVES:

1. To study the characteristics of water in Buria Nalaha, Faridabad.
2. To study the heavy metal profile of water of Buria Nalaha, Faridabad.
3. To study the growth behaviour of *Azolla* species exposed to Heavy metals Cadmium and Lead and Buria Nalaha effluents
4. To evaluate the antioxidant enzymes in *Azolla* plants under heavy metal stress.
5. To elucidate the physiological mechanism of adaptation to heavy metal toxicity.
6. To study the biosorption capacity of Cadmium and Lead by *Azolla*.

MATERIAL & METHODS

1. **Estimation of Characteristics of Water from Buria nalaha.**

   The various physical, chemical and biological parameters of Burianala will be studied. The standard methods will be followed as per APHA guidelines (1985).

2. **Estimation of heavy metals in water of Buria nalaha.**

   The profile of heavy metals in water of Burianala will be studied. The standard method for sampling as well as estimation will be followed as per APHA guidelines (1985).

3. **Study of growth parameters of *Azolla*.**

   The various growth parameters of *Azolla* under heavy metal stress of Cadmium and Lead and in presence of Burianala effluent will be studied as per the standard methods.

   Morphological Parameters

   (a) Estimation of dry weight
   (b) Estimation of fresh weight
   (c) Estimation of root growth, length and numbers.
   (d) Doubling time – Stewart & Boyd (1999), Badayos (1989)
   (e) Relative Growth Rate - Harding (2000)

   Biochemical Parameters

   a. Protein content – Lowry Method (1951)
   b. Sugar content – Hedge & Hofreiter (1962)
   c. Chlorophyll (a) and Chlorophyll (b) – Lichtentaler & Wellburn (1985)
   d. Total carotenoids - Lichtentaler & Wellburn (1985)
   e. Flavonoids – Mirecki and Teramura (1984)
   f. Proline – Bates et. al (1973)
   g. Lipid peroxidation – Heath and Packer (1968)
   h. Electrolyte leakage – Lutts et. al (1996)
   i. Super oxide radical – Chaitanya & Nathani (1994)

4. **Estimation of antioxidants enzymes.**

The behavior of various antioxidants enzymes will be studied using the following standard methods:

(a) Super Oxidase Dismutase (SOD) - Stewart and Bewley (1980)
(b) Guaicol Peroxidase - Malick & Singh (1980)
(c) Ascorbate Peroxidase (APX) - Nakano and Asada (1981)

5. Metabolic Enzymes:

a. β-Amylase - Kruger (1972)
b. Acid phosphatase - Lowry et al (1954)
c. Alkaline phosphatase - Lowry et al (1954)
d. Malate dehydrogenase - Shrikantan & Krishnamurthy (1972)
e. Glutamate dehydrogenase - Shrikantan & Krishnamurthy (1972)
f. Succinate dehydrogenase - Shrikantan & Krishnamurthy (1972)

6. Others:

a. Heterocyst frequency – Manual method

7. Estimation of Biosorption Capacity:

To study the biosorption capacity of *Azolla* for Cadmium and Lead the following parameters will be considered:

a. Effect of Particle size
b. Effect of pH
c. Effect of Temperature
d. Effect of Contact Time
e. Effect of initial concentration of Cadmium and Lead.
f. Effect of Biomass concentration

8. Desorption studies:

The desorption studies will be conducted subject to availability of facilities and time frame.

SIGNIFICANCE

Harmful effect of metallic ions on living species needs great concern with reference to industrial effluent discharge. Absence of proper treatment of effluents, lack of management policies and ineffective legislation lead to the problems.The heavy metals being stable elements are not metabolized in the body and through the food chain passes to human beings. These metals interfere with normal physiological functions of the living organisms leading to serious medication manifestations in man. Though metals have many commercial uses they are highly toxic to the body. As the conventional methods for treating industrial effluents for removing heavy metals are costly need for new economical method for the removing these toxic impurities from waste water is required. Living cells have an advantage that they work at low metal concentration, and the living biomass can multiply to produce new cells. The main disadvantage is the toxicity produced within the organism by the metal. Since these are accumulated in the body and move through the food chain therefore the use of non-living biomass is preferred over living organism to get rid of heavy metals present in high concentration.

Azolla has global distribution and grows in different conditions from dilute to polluted waterbodies. It can take up and accumulate nutrients directly from flood waters and shows high affinity for K, Fe and P. These nutrients are accumulated several times more than required by the plant and then it slowly releases these nutrients on decomposition. Based on its capability to concentrate nutrients like K,P, N and heavy metals it has also been used for treating of waste waters and is cost effective.

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